REMARKS

Claims 54, 55, 57, 75, 81, and 82 have been amended. Claims 1-53, 74 and 80 are canceled. Claims 86-99 are new. The amendments and new claims are supported throughout the application as filed, e.g., at page 2, lines 19-24; page 23, lines 8-10; page 23, lines 19-23; page 33, line 28 to page 31, line 2; and the original claims. Upon entry of this amendment, claims 54-73, 75-79, and 81-99 will be pending and under examination.

At the outset, Applicants thank Examiners Yu and Caputa for their time and thoughtful discussion during a telephonic interview conducted on July 8, 2003 with the undersigned. The substance of the interview is discussed in detail below.

Objection to the Specification

The Examiner stated that "the substitute specification filed 11-25-2002 has not been entered because it does not conform to 37 CFR 1.125(b) because: it lacks a marked up copy." Applicants do not understand this objection, since no substitute specification has been filed. Applicants respectfully ask for clarification or withdrawal of this objection.

Objection to the Information Disclosure Statement

Applicants previously submitted an Information Disclosure Statement to cite a published U.S. patent application. Applicants submit a substitute Information Disclosure Statement, Form PTO-1449, and a copy of the cited application (U.S.S.N. 09/822,161). The Examiner is respectfully requested to initial, date and return the PTO Form 1449.

Rejections under 35 U.S.C. § 112, First Paragraph

I. Enablement

Claims 54-85 were rejected under 35 U.S.C. § 112 as containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention.

This rejection has been met, in part, and is traversed, in part. Each of the Examiner's particular arguments is addressed in turn below.

A. The present claims require specific structures of TSP-2, which have been shown to work in the claimed methods

First, the Examiner stated that:

> the Office maintains that undue experimentation is required to make a fragment that retains the function cited in the instant claims because the specification does not teach the specific structures responsible for inhibiting endothelial cell migration activity in light of the art recognized unpredictability of protein chemistry.

The rejection has been met by substantially narrowing claims 54 and 81 (from which other claims depend) to require that the fragment of TSP-2 comprise *at least 10 contiguous amino acids of either a procollagen domain of TSP-2, or a type I repeat of TSP-2*. Such fragments work in the claimed methods. As discussed in the interview, the present application provides sufficient guidance for a skilled artisan to practice the full scope of the claimed methods without undue experimentation. For example, the application provides a working example showing that TSP-2 transfected cells inhibit tumor growth *in vivo* and shows that the activity of TSP-2 correlates with the ability to inhibit endothelial cell migration *in vitro* and *in vivo*. At least one fragment having a portion of a procollagen domain or a type I repeat of TSP-2 (as claimed) is disclosed, which has the same ability to inhibit endothelial cell migration *in vitro*. In addition, in the first Declaration of Michael Detmar ("the first Declaration"), filed with the response of November 19, 2002, Applicants submitted data showing that an additional fragment that falls within the claims inhibits endothelial cell migration in vitro and inhibits tumor growth in vivo. In addition, as was noted by Applicants in the response filed on November 19, 2002, the TSP-2 sequence is known, techniques for making fragments as recited in the claims are routine in the art, and the specification describes multiple assays which can be used to identify additional functional fragments.

B. The first Declaration of Michael Detmar supports enablement

The Examiner further stated that the claims are not enabled because

> the specification does not teach a method of treating a disorder using a cell expressing the various other sequences or the fragments thereof other than SEQ ID NO:2. Applicant argues that the instant claims are enabled because as disclosed in Dr. Detmar's declaration along with accompanying figures, the inventors found the specific N-terminal fragment nucleotides 213-1888 of SEQ ID NO:1 is effective. This argument is not convincing because the argument is directed to a limitation not in the claims.

This rejection has also been addressed by amending the claims to specific the regions of TSP important for activity. Claims 54 and 81 (from which other claims depend) are narrowed to require that the fragment of TSP-2 comprise at least 10 contiguous amino acids of either a procollagen domain of TSP-2, or a type I repeat of TSP-2. The fragments disclosed as effective in the first Declaration are encompassed by the limitations of the present claims. The TSP-2 fragment encoded by nucleotides 213-1888 of SEQ ID NO:1 includes the procollagen domain and three type I repeats of TSP-2.

The data provided in the specification and that described in the first Declaration of Michael Detmar demonstrate the efficacy of the TSP-2 fragments recited in the claims for *in vivo* protein therapy. Cell therapy methods, as presently claimed, work precisely because the cells expressing the TSP2 (or fragment) secrete the TSP-2 (or fragment) protein. The Examiner is directed to pages 38-41 of the specification, where experiments are described to show that the TSP-2 protein secreted from TSP-2 expressing cells exerts its effects *in trans*, on non-tumor cells such as endothelial cells of the vessel invading the tumor. In short, the cells of the presently claimed methods act as factories for the production and secretion of TSP-2 or the specific TSP-2 fragments recited in the claims, which have been found to work to reduce tumor growth. Thus, the data in the specification and the first Declaration showing that the recited protein fragments can reduce tumor growth also supports the enablement of the presently claimed cell therapy methods.

As requested by the Examiner during the interview, the data from the first Declaration of Michael Detmar, are discussed in detail below.

Figure 1 of the first Declaration is a schematic diagram of the preparation of a recombinant protein fragment of TSP-2. As outlined in the text on the figure, a fragment of TSP-2 DNA, containing nucleotides 213-1883 of human TSP-2 cDNA was PCR amplified from a human cDNA library and cloned into an expression vector. This cDNA fragment encodes the procollagen domain and three type 1 repeat domains of TSP-2. The vector was transfected in mammalian 293 EBNA cells. Stably transfected clones were selected and propagated in medium containing puromycin. To purify the TSP-2 protein fragment encoded by nucleotides 213-1883 of TSP-2 (the TSP-2 N-terminal fragment, hereafter referred to as "TSP-2/NTF"), transfected 293 EBNA cells were cultured in serum-free medium. The medium was collected from the cells every two days until the cells began to detach. The medium was filtered and proteins were precipitated with ammonium sulfate and dialyzed. Next, the TSP-2 fragment was purified by gelatin-sepharose and heparin-sepharose chromatography and dialyzed. The TSP-2 fragment was subsequently assayed *in vitro* and administered *in vivo*.

Figure 2 of the first Declaration is a graph showing the results of *in vitro* endothelial cell migration assays. These *in vitro* assays were performed essentially as described on page 39 of the specification with only minor technical changes. As shown in the graph, the greatest inhibition of migration was observed when the assay was conducted with cells that had been incubated in the presence of 1 μg/ml TSP-2/NTF protein, although inhibition was also observed with cells treated with 0.5 and 2 μg/ml TSP-2/NTF protein.

Figure 3 of the first Declaration presents results of *in vivo* treatment of tumor-challenged mice with phosphate buffered saline (PBS), endostatin, the purified TSP-2/NTF protein fragment (described above), and a fragment of the procollagen domain of TSP-2 (PC). Nude mice were injected intradermally with 2 X 10^6 A431 cells. Beginning 2 days after implantation of the A431 cells, mice were injected in the peritoneal cavity with 1 mg/kg purified TSP-2/NTF protein, PBS, endostatin, or PC. This was repeated daily for 18 days. The photo was taken 20 days after implantation of the tumor (i.e., after 18 days of treatment with the proteins). As is shown in the

photo, treatment with TSP-2/NTF protein caused marked reduction of tumor growth relative to control (PBS). PC treatment also inhibited growth relative to control. Thus, systemic administration of fragments of TSP-2 causes reduction of established tumors *in vivo*.

Figure 4 of the first Declaration is a graph showing the volume of A431 tumors over a period of 20 days in mice treated with PBS, endostatin, 1 mg/ml purified TSP-2/NTF protein, 0.01 mg/ml purified TSP-2/NTF protein, 1 mg/ml PC, and 0.1 mg/ml PC, as described for Figure 3. After 20 days, the volume of tumors in mice treated with 1 mg/ml TSP-2/NTF was approximately 700 mm^3. The volume of tumors in control mice was approximately 1200 mm^3 after the same amount of time. Treatment with TSP-2/NTF protein reduced the volume of tumors by approximately 40% over 20 days. PC treatment also reduced the volume relative to control. Figure 5 presents results of a similar experiment, in which 3 mg/kg of body weight of purified TSP-2/NTF protein was administered and compared to treatment with PBS. In this experiment, the treatment with TSP-2/NTF or PBS was initiated 4 days after implantation of the tumors. These animal models used in these experiments are art-recognized models of tumor growth.

Thus, the data described in the first Declaration shows that Applicants produced and expressed a TSP-2 protein fragment falling within the present claims, using the methods and guidance provided in the specification and other methods routine to the skilled artisan. Applicants assayed the biological function of the protein fragment *in vitro* and confirmed its activity *in vivo*. As was noted in the first Declaration, the method for testing the protein fragment of TSP-2, the A431 tumor xenograft assay, was the same tumor xenograft assays described in the specification. Clearly, use of the recited TSP-2 fragments for treatment of tumors is enabled.

The data provided in the specification and in the first Declaration of Michael Detmar support enablement for methods of using cells transfected with TSP-2 and fragments thereof because Applicants have shown that cells transfected with TSP-2 secrete TSP-2 protein, and that this secreted protein inhibits tumor growth and the growth of blood vessels. See, for example, the xenograft assays described at page 38, line 22 to page 39, line 25. In these assays, nude mice

were implanted with human epidermoid carcinoma cells of the A431 cell line, which had been transfected with TSP-2. The results of these assays show that implantation of the cells inhibited growth of tumors by more than 90% after three weeks as compared to untransfected tumors. Similar results were obtained with human malignant melanoma cells of the MeWo cell line. As discussed on page 39, lines 22-25, TSP-2 overexpression "decreased tumor angiogenesis, as shown by a decreased density of tumor vessels, as compared to control tumors."

Importantly, Applicants demonstrated that transfection of tumor cells with TSP-2 did not directly inhibit growth of the transfected tumor cells themselves, but rather, mediated biological effects *in trans*. See page 37, lines 24-27, where it states that "no significant differences in cellular morphology and growth rates on plastic culture dishes, in soft agar colonization or in spontaneous and induced apoptosis rates were observed between control transfected and TSP-2 overexpressing A431 clones." In another assay, it was demonstrated that "anchorage-independent cell growth, as determined by the ability to form colonies in soft agar, showed no significant differences between TSP-2 transfected A431 clones and control transfected A431 clones" (page 46, lines 6-8). Rather, the TSP-2 transfected tumor cells showed reduction in vascular vessel size and decreased vascular density *in vivo* (page 41, lines 10-19). That is, the TSP-2 transfected tumor cells were not themselves growth-inhibited, but rather, the growth and invasion of vascular vessels that feed the tumor was inhibited. Thus, the TSP-2 protein secreted from the transfected cells exerts its effects *in trans*, on non-tumor cells such as endothelial cells of the vessel invading the tumor, as shown in these experiments. These assays demonstrate that TSP-2 protein and TSP-2-expressing cells can be used to deliver TSP-2 protein to a subject, and that full-length and specifically claimed fragments of the protein are effective.

C. The Streit reference supports enablement

In other grounds for the rejection, the Examiner stated that the evidence provided by Applicants showing that cell therapy is effective was not convincing because "the instant specification does not disclose all of the parameters necessary for inhibiting *in vivo* tumors." The evidence provided by Applicants included data in a research article, Streit et al. (*Cancer Res.*

62:2004-2012, 2002). Streit et al. showed that treatment of mice by implantation of a biodegradable polymer mesh containing TSP-2 transfected fibroblasts inhibited the growth of three types of tumor. This grounds for the rejection is respectfully traversed.

That Streit et al. showed efficacy with cells encapsulated in a degradable polymer mesh does not necessitate this step for Applicants' claims to be enabled. Examples in the application demonstrate that cell therapy is effective without the use of a polymer mesh. The *in vivo* assays in the application describe administration of TSP-2-transfected cells. See pages 38-41 of the specification. This section of the specification makes it clear that the claimed methods work because TSP-2 transfected cells secrete functional TSP-2 protein, and this protein acts *in trans* on non-tumor cells such as endothelial cells of the vessel invading the tumor, to thereby inhibit growth of the tumor. Furthermore, the data presented in the specification and the first declaration shows that administration of TSP-2 protein also works. As Examiner Caputa pointed out, if anything, the fact that such diverse modes of administration of TSP-2 have been shown to be effective supports enablement of the claimed methods, as the claimed methods recite a mode of administration (cell therapy in general) that is of intermediate scope between protein therapy in general and the specific cell/mesh therapy of Streit.

D. The claims cover ex-vivo cell therapy methods, not *in vivo* gene therapy

In a new ground of rejection the Examiner stated that claims 54-85 are not enabled because:

> the specification fails to disclose *in vivo* demonstration of cancer treatment using the active steps of the invention. The *in vivo* data demonstrated in Fig. 3 of the instant application along with the attached figures...use gene therapy as the active step, not the active steps claimed in the instant invention...The guidance and the example in the specification is administering a nucleic acid molecule and there is no example or guidance about administering a cell expressing a protein. Streit et al...teach consideration necessary for successful cell-based therapy is different from the necessary consideration for successful gene therapy.

This rejection is respectfully traversed. The present claims do not, on their face, cover in-vivo gene therapy and are not intended to do so. The claims are directed to methods of treating

angiogenesis-dependent tumors by administering cells expressing TSP-2 or a functional fragment thereof. Furthermore, the specification does teach methods of treatment using cells expressing TSP-2. See, for example, the xenograft assays described at page 38, line 22, to page 39, line 25. In these assays, nude mice were implanted with human epidermoid carcinoma cells of the A431 cell line, which had been transfected with TSP-2. The results of these assays show that TSP-2 expression inhibited growth of tumors by more than 90% after three weeks as compared to untransfected tumors. Similar results were obtained with TSP-2-transfected human malignant melanoma cells of the MeWo cell line. Rather, the TSP-2 transfected tumor cells showed reduction in vascular vessel size and decreased vascular density in vivo (page 41, lines 10-19). That is, the TSP-2 transfected tumor cells were not themselves growth-inhibited, but rather, the growth and invasion of vascular vessels that feed the tumor was inhibited. Thus, the TSP-2 protein secreted from the transfected cells exerted effects in *trans*, on non-tumor cells such as endothelial cells of the vessel invading the tumor, as shown in these experiments. Thus, Applicants have shown that administration of TSP-2 transfected cells inhibits tumor growth in animals.

E. The claimed methods work to inhibit tumor growth

The Examiner's next point in the enablement rejection is that cancer therapy is unpredictable. This basis for the rejection is respectfully traversed. While it may be true that cancer therapy is *generally* unpredictable, Applicants are not claiming treating tumors with any and all methods generally. The present claims relate to a specific method, namely administering cells expressing TSP-2 or specifically recited fragments thereof. Applicants have shown, in multiple examples, that the methods work to inhibit tumor growth *in vivo*. A skilled artisan, using the guidance provided in the specification, can readily perform the claimed methods. Nothing more is required.

The Examiner cited five references (discussed in more detail below) that discuss the difficulties of cancer therapy. Few of these references discuss anti-angiogenic therapy, much

less therapy with cells expressing TSP-2. The limitations in cancer therapies discussed in the cited references do not apply to the claimed methods.

Gura (1997) is concerned primarily with compounds identified by random screening, rather than therapeutics derived from naturally occurring proteins that regulate tumor growth. Jain (1994) examines the methods by which tumors resist penetration by drugs. Jain, however, does not teach that anti-angiogenic therapy is unpredictable. In fact, Jain teaches that such therapy may be a solution to the problem of drug penetration, thus supporting enablement of the claimed methods. See page 65, paragraph 6, where Jain states "alternatively, if a tumor's vascular system could be destroyed completely, no drug would have to extravasate or cope with the interstitium. The persistent, total lack of nourishment would be expected to starve and eventually kill tumor cells." Applicants have demonstrated this very effect *in vivo*. See, for example, page 40, lines 11-13, where necrosis of TSP-2-expressing tumors is described. Curti (1993) discusses chemotherapy and therapy with monoclonal antibodies, which is a completely different type of molecule than TSP-2. Curti does not discuss anti-angiogenic therapy such as that of the claimed methods. Curti does, however, state that "the key to understanding the unique properties of a tumor's physiology requires focus on the tumor vasculature and interstitial space" (at page 30, paragraph 3). Indeed, the claimed methods target the vasculature of a tumor, as suggested by Curti. Hartwell et al. (1997) discusses the utility of genetically tractable organisms in searching for cancer targets. Curti also discusses the problem of extrapolating data from non-mammalian organisms, such as yeast, to mammalian systems when designing anti-cancer therapeutics. These issues do not support non-enablement of the claimed methods, as the claimed methods have been shown to work *in vivo* on a mammalian model system. Bellone et al. (2000) is cited as teaching that DNA and protein therapeutics provide different, unpredictable results. Bellone et al. is concerned with immunological anti-cancer strategies, in which an animal's immune system is stimulated to react to tumor tissue. These strategies are also unrelated to the presently claimed methods of treatment, which relate to a different mechanism, namely angiogenesis.

The presently pending claims are directed to treatment of angiogenesis-dependent tumors. As discussed in the enclosed Second Declaration of Michael Detmar under 37 C.F.R. § 1.132 (copy enclosed), originally filed in the parent application in an Amendment of July 30, 2003, one of ordinary skill in the field of angiogenesis would understand that inhibition of one of the steps of angiogenesis would inhibit growth of any angiogenesis-dependent tumor. As such, one of ordinary skill would understand, based on the disclosed activities of TSP-2 and the data provided in the specification, that TSP-2-expressing cells and cells expressing the recited TSP-2 fragments can be used in methods of treating disorders characterized by tumors.

In summary, the claims have been narrowed to recite explicit limitations with regard to required TSP2 structures. Given the explicit limitations of the claims, the extensive guidance provided in the specification, including multiple *in vivo* experiments, combined with the knowledge and high level of skill in the art, a skilled artisan could perform the claimed methods without undue experimentation. Accordingly, Applicants respectfully request that the rejection be withdrawn.

II. Written Description

Claims 81 and 82 are rejected under 35 U.S.C. § 112 as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the art that the inventors had possession of the claimed invention. The Examiner stated that "Applicant argues that the instant claims are drawn to at least 95% identical to SEQ ID NO:2 but this is not convincing because Applicant argues with a limitation not present in the claims." Claims 81 and 82 were added by the amendment of November 19, 2002. Therefore, Applicants discussion of written description was limited to the claims under examination at that time (i.e., claims 54-80). A basis for lack of written description support for claims 81 and 82 has not been provided. Therefore, Applicants ask for clarification or withdrawal of this rejection.

Rejections under 35 U.S.C. § 112, First Paragraph

Claim 57 is rejected for reciting "aid". This typographical error has been corrected to recite "acid". Claim 81 is rejected for reciting "the nucleotide sequence of SEQ ID NO:2." This has been corrected to recite "the nucleotide sequence of SEQ ID NO:1."

In view of the foregoing, Applicants ask the rejection of the claims be withdrawn.

Enclosed is a Petition for Extension of Time. A check in the amount of $930 is enclosed for the fees. Please apply any other additional charges or credits to deposit account 06-1050.

Respectfully submitted,

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